CAUSAL INFLUENCES OF AIRBORNE FUNGI AND OTHER FACTORS ON SYMPTOMS OF RESPIRATORY ALLERGIES

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ABSTRACT
The aerospora indoors in 15 residences in Kitchener-Waterloo, ON, Canada was studied. Allergenco samplers were used in the study. The results showed that indoor total fungal spores, indoor *Aspergillus/Penicillium* and the age of the residences have significant effects on symptoms. Dampness, age of the patient, and cleanness have significant direct and indirect effects on symptoms. Dampness was the most important factor, showing a strong causal influence on symptoms. However, the exact role of dampness in relation to symptoms needs further research.

INDEX TERMS
Airborne fungi, Causal effect, Dampness, Path analysis, Respiratory symptom.

INTRODUCTION
The indoor environment is a complex ecosystem (Tobin *et al.*, 1987). Risk assessment in indoor environments is an important health issue. The importance of indoor fungi is becoming increasingly understood by the public (Scott, 2001). In developed countries, the majority of the people spend more than 90% of their time indoors, and thus experience long exposure to common airborne pollutants, which have potentially adverse health effects.

Fungi in the residence are a world-wide phenomenon (van Bronswijk *et al.*, 1986). The significance of airborne fungi in inciting allergic reactions in human beings has long been realized (Misra and Jamil, 1991). Although a number of studies have been conducted on the subject of airborne fungi indoors, most originated from a medical perspective and were generally weak with respect to numerical and/or taxonomic data (Tobin *et al.*, 1987).

The allergenic effects of airborne fungal spores have long been recognized by medical professionals and by the public. Research on correlations between airborne fungi and symptoms of allergic respiratory diseases has been reported, but correlations do not necessarily mean causal relationships, and it is far more important to understand the causal relationships than the correlations. The objective of this study is to examine the causal relationships between the composition and population of indoor airborne fungal spores, and the symptoms of respiratory allergies.

MATERIALS AND METHODS
Fifteen residences in the Kitchener-Waterloo area, ON, Canada were selected as indoor air-sampling sites. Twelve of the 15 residences housed allergic patients. In each residence air

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samples were taken from six sampling sites: living room, kitchen, bedroom, bathroom, family room and outside, except in the case of apartments, where five sites were chosen, excluding family room. Samples were taken once a month at each residence, at a time between 1:00 pm and 9:30 pm. In each room or site, three 10 min samples were taken with a Samplair-MK1 or -MK2 particle sampler. Samplers were put on a table or a night table 50 - 80 cm in height during indoor sampling. For outdoor sampling the sampler was on the ground. Three samplers drawing 9.0, 15.0 and 15.5 L of air/min were used in this study. Samplers were assigned to the rooms randomly at each sampling date to avoid sampler effect. In ten residences, the sampling continued for at least one year; in the remainder from six to nine months, because the houses were sold or the patients withdrew from the study. At each sampling site and date, temperature and relative humidity (RH) were recorded. At the same time, the presence of plants, pets and carpets in sampling rooms were noted.

The patients were chosen by an allergist. Every patient was proved to be allergic to moulds by allergy tests. The patients were asked to fill in an initial questionnaire and an ongoing allergy dialy diary. The questionnaire included questions about personal data and habits, disease history, symptoms, residence characteristics, residence history and possible dampness/mould problems. The allergy diary included 18 symptoms of allergic respiratory diseases on a 4-level scale (0-absent, 1-mild, 2-moderate and 3-severe) (for detailed information on symptoms, Li and Kendrick, 1995).

The slides used in sampling were coated with a thin layer of a mixture of 90% vaseline and 10% high melting point wax (by weight) and subsequently mounted with polyvinyl lactophenol. All fungal spores from the samples were counted and identified under the 40x or 100x objective of a Nikon light microscope equipped with phase contrast optics.

The distribution of the data were checked for homogeneity of variance, indoor *Aspergillus/Penicillium*, indoor *Cladosporium*, disease history, outdoor unidentified fungal spores and total fungal spores were log-transformed to improve normality. The causal relationships of indoor fungal spores with environmental factors were analyzed with AMOS^{TM} 3.1 software (Arbuckle, 1992). The relationships of allergenic symptoms with airborne fungal spores and environmental factors were examined by multiple regression on SYSTAT and path analysis on AMOS^{TM} 3.1. The correlation matrix was used as a data file for all path analysis to circumvent the limitations of the AMOS^{TM} 3.1 software.

RESULTS

Multiple regression analysis of symptoms as dependent variable, with *Alternaria*, patient age, cat, cleanness, dampness, filter, gender of the patients, indoor number of genera, house age, humidifier, heating system, indoor *Leptosphaeria*, indoor *Aspergillus/Penicillium*, history of illness, outdoor hyphal fragments, outdoor unidentified spores, total fungal spores, outdoor *Cladosporium*, and smoking as independent variables, showed that 62.3 % of the variance could be explained by the regression model (Table 1). All the independent factors were statistically significant. Variables being significant in this analysis and Pearson Correlation analysis (Li and Kendrick, 1996) were history of illness (Coeff = 0.747), indoor total spores (Coeff = 0.281), indoor *Aspergillus/Penicillium* (Coeff = 0.049), cleanness (Coeff = -0.257), dampness (Coeff = 0.286), filter (Coeff = -0.488), gender of the patients (Coeff = -0.594: severer to male), humidifier (Coeff = -0.618), smoking (Coeff = 0.246) and forced air heating system (Coeff = 0.362).

According to the results of the exploratory data analysis and our knowledge of allergy, a model was developed which combined both direct and indirect causal factors with indoor total fungal spores (LTO), indoor *Aspergillus/Penicillium* (LAP), Dog (DO), cleanness (CL), Dampness (DP), age of the residences (HA) and age of the patients (AG) as explanatory variables possessing direct effects and with dog, cleanness, dampness and age of the patients as explanatory variables having indirect effects on the symptoms of allergenic respiratory diseases (LS) (Figure 1). The variables chosen in the model were significant in both correlation and multiple regression analyses. This causal model is a recursive system with five exogenous explanatory variables, three jointly dependent variables, and random error variables (e). The error variables are composed of all residual causes associated with the dependent variables. The equations for the model are:

$$LTO=p_{lto\text{-}dp}DP+p_{lto\text{-}ag}AG+e_{lto} \quad (1)$$

$$LAP=p_{lap\text{-}dp}DP+p_{lap\text{-}cl}CL+p_{lap\text{-}do}DO+e_{lap} \quad (2)$$

$$LS=p_{ls\text{-}lto}LTO+p_{ls\text{-}lap}LAP+p_{ls\text{-}ag}AG+p_{ls\text{-}ha}HA+p_{ls\text{-}dp}DP+p_{ls\text{-}cl}CL+p_{ls\text{-}do}DO+e_{lap} \quad (3)$$

Table 1. Standard multiple regression between symptoms and allergen-related factors. n = 769, $r^2 = 0.615$.

VARIABLE	COEFF*	SE	SC	TOLERANCE	T	P
Dependent Variable						
LS						
Independent Variable						
CONSTANT	0.922	0.384	0.000	.	2.402	0.017
LAP	0.049	0.013	0.102	0.7134603	3.783	.17E-03
LHI	0.747	0.048	0.705	0.2532493	15.641	.10E-14
LOH	0.057	0.016	0.090	0.7867397	3.502	.49E-03
LOU	-0.086	0.016	-0.149	0.6661471	-5.364	.11E-06
LTO	0.281	0.037	0.344	0.2486342	7.561	.12E-12
A	-.375899E-03	.152011E-03	-0.067	0.6975448	-2.473	0.014
AG	0.016	0.004	0.267	0.1543787	4.627	.44E-05
CA	0.195	0.051	0.097	0.7934874	3.793	.16E-03
CL	-0.257	0.073	-0.218	0.1334324	-3.513	.47E-03
DP	0.286	0.097	0.127	0.2780448	2.961	0.003
F	-0.488	0.129	-0.213	0.1603190	-3.770	.18E-03
GD	-0.594	0.130	-0.272	0.1449864	-4.572	.56E-05
GE	-0.055	0.017	-0.145	0.2482894	-3.176	0.002
HA	-0.049	0.003	-0.570	0.3345192	-14.531	.10E-14
HF	-0.618	0.080	-0.284	0.3771916	-7.682	.49E-13
HS	0.362	0.178	0.164	0.0793894	2.038	0.042
L	-.788490E-04	.185682E-04	-0.103	0.8695841	-4.246	.24E-04
OC	-.288897E-04	.101191E-04	-0.089	0.5311352	-2.855	0.004
SK	0.246	0.097	0.086	0.4415271	2.530	0.012

*COEFF= coefficient, SE=standard error, SC=standard coefficient, LAP=logged *Aspergillus/Penicillium*, LHI=logged history of illness, LOH=logged outdoor hyphal fragments, LOU=logged outdoor unidentified spores, LTO=logged total fungal spores, A=*Alternaria*, AG=age, CA=cat, CL=cleanness, DP=dampness, F=filter, GD=gender, GE=genera, HA=house age, HF=humidifier, HS=heating system, L=*Leptosphaeria*, OC =outdoor *Cladosporium*, SK=smoking.

The path coefficient is represented by p, the subscript $p_{lto\text{-}dp}$ means the path from dampness to total fungal spores. Path coefficients correspond to the standardized partial regression coefficients of multiple regression, so they represent the influence of one variable with all other variables statistically held constant (Li, 1975).

The model described 38.3% of the variance in the symptoms. The path coefficients in this model were all significantly different from zero except $p_{ls\text{-}do}$ and $p_{lto\text{-}dp}$. This means that indoor total fungal spores, indoor *Aspergillus/Penicillium* and the age of the residences had significant direct effects on symptoms, and the age of the patients ($p_{ls\text{-}ag}$ = -0.239, dampness ($p_{ls\text{-}dp}$ = 0.354) and cleanness ($p_{ls\text{-}cl}$ = 0.102) had significant both direct and indirect effects on symptoms. Dog ($p_{lap\text{-}do}$ = -0.094) had a significant indirect effect through *Aspergillus/Penicillium* on symptoms. Among the factors, the causal effect of indoor *Aspergillus/Penicillium* was stronger than those of total fungal spores on symptoms ($p_{ls\text{-}lap}$=0.196, $p_{ls\text{-}lto}$=0.089), which were significantly positive. Dampness was the most important factor with a direct effect on symptoms ($p_{ls\text{-}dp}$=0.354). Dampness had a strong significant negative effect on the health of allergenic patients. Increasing age of the residences and decreasing age of the patients ranked second and third and had a negative effect on symptoms ($p_{ls\text{-}ha}$=-0.248, $p_{ls\text{-}ag}$=-0.239). The effect of cleanness fell between that of indoor *Aspergillus/Penicillium* and indoor total fungal spores ($p_{ls\text{-}cl}$=0.102).

The overall fit of the causal model was good: χ^2=2.526, df=3, P=0.471. Examination of the solution of the path analysis indicated that the parameter estimates (unstandardized path coefficients) had acceptable standard errors. The modification indices showed that the model would not be considerably improved by adding other path effects between the factors.

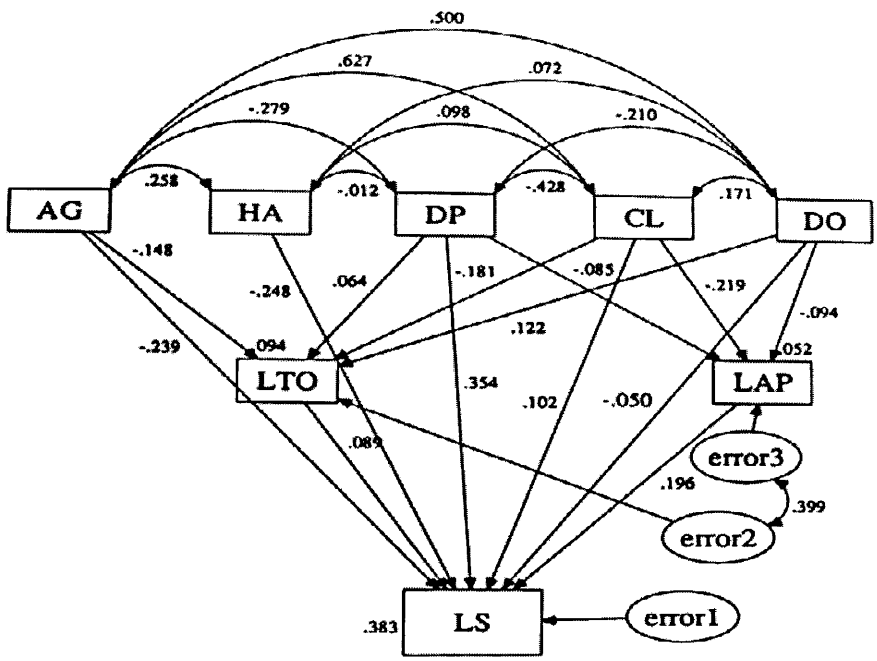

Figure 1. Causal influences of airborne fungal spores and environmental factors indoors on the symptoms of allergenic respiratory diseases. AG=age of patients, CL=cleanness, DO=dog, DP=dampness, HA=age of residences, LAP=logged indoor *Aspergillus/Penicillium*, LS=logged symptoms of allergic respiratory diseases, LTO=logged indoor total spores.

DISCUSSION

Aspergillus/Penicillium showed a statistically significant causal effect on symptoms. It is necessary to understand the potential differences of allergenicity among congeneric species. For example, the allergenicities of *Aspergillus niger* v. Tiegh and *A. ruber* (Konig. et al.) Thom et Church differ by a factor of more than 10 times (van Bronswijk et al., 1986). The causal impact of total spores on symptoms meant that some of the airborne fungi in the total spores, especially those from indoor sources, had adverse effects on human health. The base level of indoor fungal spore counts may partly be caused by unnoticeable fungal micro-colonies that develop on temporarily wet surfaces (Pasanen et al., 1992), and there may also be other indoor fungal spore sources (Lehtonen et al., 1993). Some studies have indicated that there is not always a good correlation between allergenic symptoms and total airborne fungi (Tobin et al., 1987). Allergenic problems may occur at concentrations of airborne fungal spores not associated with formation of visible fungal colonies (van Bronswijk et al., 1986).

Dampness (excessive humidity or water intrusion) has an adverse effect on human health (Platt *et al.*, 1989). Numerous studies have established high correlations between dampness and self-reported respiratory symptoms (Strachan *et al.*, 1990; Platt *et al.*, 1989; Dekker *et al.*, 1991). According to the model in the present study, dampness was the most important factor correlating to symptoms of respiratory diseases, but dampness itself is not a causal agent of any negative effects on human health. It appeared to be dampness-induced changes in the indoor biota that caused ill-effects to humans. It is well known that in sufficiently moist conditions almost any material can become mouldy (Pasanen *et al.*, 1992b). These authors found that some indoor airborne fungal spores were derived from unnoticeable fungal microcolonies, which may develop on temporarily wet surfaces. Home dampness is a potential risk factor for respiratory disease, especially for allergy, in part through the effects of microorganisms, such as fungi, dust mites and bacteria that thrive in damp environments (Brunekreef *et al.*, 1989). An increase in bacteria can result in fungal growth which in turn can provide the food for mites. These organisms as well as some of their byproducts can be direct or indirect allergens (Tobin *et al.*, 1987).

Although dog dander is an allergen for some patients, this was not the case for most patients in the present study. Dogs caused an increase of total spores indoors, perhaps because the dog was a carrier of fungal spores from outdoors to indoors (Broadbent, 1960). Cleanness seems to cause an increase of symptoms, but the fact is that once a patient was diagnosed as hypersensitive to allergens, he/she was advised to keep the residence clean to maintain the allergens at lower levels. Most patients followed this advice.

The patients in newer dwellings experienced worse episodes of allergenic respiratory diseases in the present study. This may have been due to the fact that the concentration of volatile organic compounds from carpets, wallpaper, paints, and pressed-wood products were still high in newer dwellings, especially in dwellings constructed within the last three years. Two houses and a town house built in past three years were included in the study. The volatiles may have adverse effects on health, depending on the exposure time and the types of volatiles (Hines *et al.*, 1993). Children are more vulnerable to allergic asthma than adults (Dekker *et al.*, 1991). Since no older patients were chosen for the study, it was understandable why age of patients influenced symptoms negatively.

Field studies only describe relationships between mold exposure and health effect (Rylander, 1999). It is difficult to relate the symptoms to any single allergenic factor. Frequently symptoms were triggered by interactions of multiple factors. It is unusual for a patient to be

allergic to only a single allergen, and he/she is generally allergic to several different allergens at different allergenic response levels.

CONCLUSION AND IMPLICATIONS

Dampness and *Aspergillus/Penicillium* showed direct causal influence on symptoms, but this causal influence cannot be interpreted as meaning that dampness is itself a causal agent. Path Analysis is a good method for revealing the causal effects of the factors concerned in two aspects: first as an exploratory analysis to single out factors having potential causal impacts on symptoms, and to generate hypotheses for further research; and second, as a method to assess the relative importance of different causal pathways of allergenic factors, once a tentative model has been structured. However the causality needs clinical studies to confirm.

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